A SEROLOGICAL ANALYSIS OF THE SYSTEMATIC RELATIONSHIP OF THE BRACHYURAN CRAB GERYON QUINQUEDENS

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The taxonomic relationship of the brachyuran crab *Geryon quinquedens* is open to question. Rathbun (1918) places the species in a sub-family, the Carcinoplacinae, of the family Goneplacidae. Bouvier (1940), on the other hand, lists Geryon as a genus in the family Xanthidae. Dr. Waldo Schmitt, U. S. National Museum, has indicated (personal communication to Boyden in 1935) that morphological studies do not yield a precise placement of this species in the families of the Brachyura.

Boyden (1943) conducted the first serological tests with the serum of *Geryon quinquedens*. His tests, involving among others an anti-Geryon and an anti-Menippe serum, showed reciprocal reactions which indicated as high a degree of relationship between Geryon and Menippe, the latter a member of the family Xanthidae, as either one of these showed with other brachyuran Crustacea.

This paper presents additional serological data on the systematic position of Geryon as revealed through the precipitin reaction.

Materials and Methods

Through the facilties of the Serological Museum of Rutgers University, New Brunswick, New Jersey, it has been possible to assemble serum samples of species of Crustacea which are widely scattered geographically.[1] A list of the species whose sera were used in this report to make a serological analysis of the taxonomic position of Geryon is given in Table I.

Table 1

Table showing the species whose sera were compared by serological means in this paper

Species	Source
Geryon quinquedens Smith	Tortugas, Florida, U. S. A.
Menippe mercenaria (Say)	Unknown
Panopeus herbstii Milne Edwards	Unknown
Eriphia spinifrons (Herbst)	Naples, Italy
Callinectes sapidus Rathbun	Delaware Bay, New Jersey, U. S. A.
Portunus puber Linnaeus	Dinard, France
Cancer pagurus Linnaeus	Dinard, France
Pachygrapsus marmoratus Fabricius	Naples, Italy
Ocypode albicans Bosc	Tortugas, Florida, U. S. A.
Maia squinado Rondalet (Herbst)	Plymouth, England

[1] The author in his capacity as an officer of the Serological Museum in the summer of 1948 collected samples of sera from European Crustacea at marine biological stations in France and Italy. Other sera indicated in Table I were either collected personally by the author or provided from the stocks of the Serological Museum.

The methods for preparing rabbit antisera against the Crustacea hemocyanins and the technique for performing turbidimetric titrations of these antisera with antigens have been described in detail elsewhere (Boyden, 1942; Boyden and De Falco, 1943; Leone, 1949). In brief, the antisera were prepared in rabbits which received intravenously one or two series of four doubling injections of antigen. Serological comparisons were made by measuring with a Libby photronreflectometer (1938) the precipitates which developed when antigens and antibodies were mixed in varying proportions. To each dilution of a doubling dilution series of antigen was added a constant amount of antiserum. The entire reaction range from antigen excess to antibody excess was covered. The antigen-antibody mixtures were incubated for twenty minutes at 38° C. The precipitates which formed in the mixtures were then read in the turbidimeter. The areas of the resulting curves were used as a statistical index of the amount of reactivity between the particular antigen and antiserum being tested.

Experimental Results

The serological data are summarized in Table II.

Table II

Table showing serological relationships among brachyuran Crustacea. Values are given in percentages. Comparative values are read in vertical columns. Family names are given under each scientific name. The homologous test is identified as the 100 per cent reaction. Heterologous antigens react with the antisera in proportion to their degree of serological correspondence to the homologous antigen. Relationship values within 5 per cent of one another are considered to be of the same order of magnitude.

Antigens	Antisera								
	Geryon quinquedens	*Menippe mercenaria* (Xanthidae)	*Eriphia spinifrons* (Xanthidae)	*Callinectes sapidus* (Portunidae)	*Portunus puber* (Portunidae)	*Cancer pagurus* (Cancridae)	*Pachygrapsus marmoratus* (Grapsidae)	*Ocypode albicans* (Ocypodidae)	*Maia squinado* (Majidae)
Geryon quinquedens	100	35	28	59	37	45	64	14	14
Menippe mercenaria (Xanthidae)	39	100	28	54	35		64	34	13
Eriphia spinifrons (Xanthidae)	37	35	100	51	37	46	65	32	13
Panopeus herbstii (Xanthidae)	40	36	26	51	37		65	39	14
Callinectes sapidus (Portunidae)	36	36	23	100	37	48	54	30	21
Portunus puber (Portunidae)	34	35	21	62	100	51	55	30	20
Cancer pagurus (Cancridae)	26	27	18	40	25	100	68	33	16
Pachygrapsus marmoratus (Grapsidae)	16	15	15	25	20	40	100	35	16
Ocypode albicans (Ocypodidae)	15	12	10	21	25	35	64	100	13
Maia squinado (Majidae)	5	12	10	21	25	22	25	5	100

The data obtained with the anti-*Geryon quinquedens* rabbit serum reveal that:

(a) Of all the heterologous antigens, those of the Xanthidae and Portunidae react most. There is no significant difference in the reactivity of the species of these two families with the anti-Geryon serum.
(b) The serum of the cancrid crab reacted only slightly less than the sera of Portunidae and Xanthidae.
(c) Sera of the Grapsidae and Ocypodidae react with the anti-Geryon serum equally but still less than the above families.
(d) Of all the families tested, the representative of the Majidae gave the least amount of reactivity with the anti-Geryon serum.

The results obtained using the anti-*Menippe mercenaria* rabbit serum and the anti-*Eriphia spinifrons* rabbit serum were virtually the same for the heterologous antigens as the results obtained with the anti-Geryon rabbit serum. The serum of Geryon, moreover, reacted as strongly with these two antisera as did any other heterologous antigen tested. In view of this fact, and inasmuch as Menippe, Eriphia and Panopeus are all members of the family Xanthidae, the serological affinities of Geryon could place it in, or close to, this family.

The extent of the reaction of the species of the family Xanthidae, including Geryon, with antisera made against the sera of species of the Portunidae would indicate a close relationship between the two families. In fact, the heterologous Portunidae antigen did not react to any greater extent with an anti-Portunidae serum than did the sera of the Xanthidae. The antigen next most reactive with the anti-*Callinectes sapidus* rabbit serum was from the family Cancridae. These were followed by sera of the Ocypodidae, Grapsidae and Majidae, all equally reactive with the anti-Portunidae serum. The anti-*Portunus puber* rabbit serum was less discriminating than the anti-*Callinectes sapidus* rabbit serum. The former antiserum arranged the antigens which were tested into two principal groups, the one containing the Portunidae and Xanthidae, the other containing the Cancridae, Grapsidae, Ocypodidae and the Majidae. The latter anti-portunid serum set apart the other portunid crab from the Xanthidae, and also separated the Cancridae from the other families tested. It is interesting to note in connection with this antiserum that the proteins of Geryon reacted as much as the proteins of the heterologous portunid crab. This is unusual in view of the discriminating capacity of the antiserum. On the basis of this serological comparison, Geryon could be placed in the Portunidae rather than in the Xanthidae.

The anti-*Cancer pagurus* rabbit serum reacted most strongly with antigens from species in the families Portunidae and Xanthidae. Geryon's serum reacted as much with this antiserum as did the xanthid serum. Sera from species representing the families Grapsidae and Ocypodidae reacted somewhat less than those of the portunid and xanthid crabs. The antigens of *Maia squinado* reacted least of all with the anti-*Cancer pagurus* rabbit serum.

The anti-*Pachygrapsus marmoratus* serum grouped as equally reactive the sera of the species of the Cancridae, Xanthidae, and Ocypodidae. Sera of species from the Portunidae reacted slightly less, and the serum from the family Majidae the least.

Except for the sera of Maia and Geryon, which reacted less than the others, all the heterologous antigens reacted to approximately the same extent with the anti-*Ocypode albicans* rabbit serum. The writer can offer no explanation why the serum of Geryon should be distinguished in this manner from the sera of the xanthid and portunid crabs.

The anti-*Maia squinado* rabbit serum was unable to distinguish preferentially any of the heterologous antigens.

Discussion

Bouvier's classification (1940) of *Geryon quinquedens* is more generally confirmed by serological tests in this report than is the classification of Borradaile (1907) as copied and adapted by Rathbun (1918, 1937). Geryon serologically appears to be more like species in the families Xanthidae and Portunidae than like the species of any other of the families tested. Unfortunately, at the present writing, there is no true goneplacid serum available with which to compare the serum of Geryon or the sera of xanthid and portunid crabs. The relationship of Geryon to the Goneplacidae cannot be conclusively settled until these comparisons are made. The unlikelihood of obtaining the sera of the Goneplacidae, which would make the critical serological analysis possible, has prompted me to submit such data as are available. It is difficult to believe, however, that Geryon could exhibit more serological correspondence to the sera of the Goneplacidae than it has with those of the Xanthidae and Portunidae unless the sera of the Goneplacidae have an amount of serological correspondence to the Xanthidae and the Portunidae similar to that exhibited by Geryon.

The position of the family Cancridae as revealed through the serum of *Cancer pagurus* is interesting. When analyzed with the more discriminating antisera made against the sera of species from the tribe Brachyrhyncha such as the anti-Menippe and the anti-Callinectes sera, the antigens of Cancer are separated from those of species in Bouvier's tribe Corystoidea. Similarly, the anti-*Cancer pagurus* rabbit serum indicated that the xanthid and portunid antigens were more similar to those of Cancer than were the antigens of the grapsid and ocypodid crabs. In contrast to this the less discriminating anti-xanthid and anti-portunid rabbit sera, namely anti-Eriphia serum and anti-Portunus serum, classified the Cancer antigens as being of about the same order of reactivity as the sera of the grapsid and ocypodid crabs. Antisera against the proteins of *Pachygrapsus marmoratus* and *Ocypode albicans* did not give any greater reaction with the serum of Cancer than they did with the sera from species in the other families. The serological data indicates then that with appropriate antisera the proteins of *Cancer pagurus* can be shown to be more similar to the xanthid and portunid crabs than to the grapsid and ocypodid crabs.

Whether or not Bouvier was justified in placing Cancer in the tribe Corystoidea instead of the tribe Brachyrhyncha is a matter of opinion. The criteria which are used to establish different systematic categories are often debatable. In the case of serological data, homologous proteins of two species from different yet closely related tribes might exhibit greater serological correspondence than the homologous proteins of two widely separated species within a single tribe. This is not inconsistent with good systematics.

The anti-Grapsidae and anti-Ocypodidae rabbit sera did not give a greater reaction with the antigens of Geryon over the other heterologous antigens tested. The family Goneplacidae was classified by Bouvier with the Grapsidae and Ocypodidae in the group Catometopa. If this classification is correct, then Geryon is more correctly placed as a genus in the family Xanthidae or in the family Portunidae. However, as indicated above, exact serological relationship of Geryon to the Goneplacidae still remains to be determined.

Summary

1. Serological tests have revealed that the serum of the brachyuran crab *Geryon quinquedens* has a greater degree of correspondence to the sera of species in the families Xanthidae and Portunidae than to the sera of species in the families Cancridae, Ocypodidae, Grapsidae and Majidae.

2. Of the species which were tested serologically, those in the families Xanthidae and Portunidae have the closest relationship. The family Cancridae is closer to the Xanthidae and Portunidae than any other of the families tested.

3. The families Grapsidae, Ocypodidae, and Majidae are quite distinct from one another, and from the other families tested. The family Majidae is serologically least like any of the families tested.

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